● INVITED REVIEW

MicroRNAs in Parkinson's disease and emerging therapeutic targets

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Abstract
Parkinson's disease (PD) is the second most common age-related neurodegenerative disorder, with the clinical main symptoms caused by a loss of dopaminergic neurons in the substantia nigra, corpus striatum and brain cortex. Over 90% of patients with PD have sporadic PD and occur in people with no known family history of the disorder. Currently there is no cure for PD. Treatment with medications to increase dopamine relieves the symptoms but does not slow down or reverse the damage to neurons in the brain. Increasing evidence points to inflammation as a chief mediator of PD with inflammatory response mechanisms, involving microglia and leukocytes, activated following loss of dopaminergic neurons. Oxidative stress is also recognized as one of the main causes of PD, and excessive reactive oxygen species (ROS) and reactive nitrogen species can lead to dopaminergic neuron vulnerability and eventual death. MicroRNAs control a range of physiological and pathological functions, and may serve as potential targets for intervention against PD to mitigate damage to the brain. Several studies have demonstrated that microRNAs can regulate oxidative stress and prevent ROS-mediated damage to dopaminergic neurons, suggesting that specific microRNAs may be putative targets for novel therapeutic strategies in PD. Recent human and animal studies have identified a large number of dysregulated microRNAs in PD brain tissue samples, many of which were downregulated. The dysregulated microRNAs affect downstream targets such as *SNCA, PARK2, LRRK2, TNFSF13B, LTA, SLC5A3, PSMB2, GSR, GBA, LAMP-2A, HSC.* Apart from one study, none of the studies reviewed had used agomirs or antagomirs to reverse the levels of downregulated or upregulated microRNAs, respectively, in mouse models of PD or with isolated human or mouse dopaminergic cells. Further large-scale studies of brain tissue samples collected with short postmortem interval from human PD patients are warranted to provide more information on the microRNA profiles in different brain regions and to test for gender differences.

Key Words: *Parkinson's disease; brain tissue; microRNAs; therapeutic targets; humans; animal models*

Introduction

Parkinson's disease (PD) is the second most common age-related neurodegenerative disorder, affecting an estimated 7–10 million people worldwide (Valente et al., 2012). While less than 0.1% are affected among persons < 60 years of age, prevalence increases to 1–2% in those aged > 60 years (Shulman et al., 2011) and 2–3% in those aged > 80 years (de Lau and Breteler, 2006). The clinical main symptoms are caused by a loss of dopaminergic neurons in the substantia nigra, corpus striatum and brain cortex (Braak et al., 2004; Shulman et al., 2011). Patients exhibit a range of clinical symptoms, with the most common affecting motor function including resting tremor, rigidity, akinesia, bradykinesia and postural instability (Winklhofer and Haass, 2010). Non-motor symptoms are often an integral part of the disease and some of them, such as depression, anxiety and hyposmia, can precede the onset of Parkinsonism (Ceravolo et al., 2010). Over 90% of patients with PD have sporadic PD, also known as idiopathic PD (Thomas and Beal, 2007; Valente et al., 2012), and occur in people with no known family history of the disorder. Widespread aggregates of α-synuclein protein in the substantia nigra, together with the presence of cytoplasmic α-synuclein aggregates called Lewy bodies and α-synuclein filaments called Lewy neurites in degenerating neurons, are a pathological hallmark of sporadic PD (Winklhofer and Haass, 2010). Elevated levels of α-synuclein mRNA in substantia nigra dopamine neurons have been observed in sporadic PD (Shulman et al., 2011). Although the causes of these cases remain unclear, sporadic PD likely results from a complex interaction of environmental/acquired and genetic/inherited factors (Nuytemans et al., 2010). A small proportion of cases can be attributed to genetic factors with an autosomal or recessive pattern of inheritance and are sometimes referred to as familial Parkinson's disease. Mutations in *SNCA, PARKIN, UCHL-1, PINK1, DJ-1* and *LRRK2* are the origin of familial cases of Parkinson's disease, although they account for only 5–10% of patients.

MicroRNAs are abundant, endogenous, short, noncoding RNAs that act as important post-transcriptional regulators of gene expression by binding to the 3'-untranslated region (UTR) of their target mRNAs, thereby interfering with translation or causing destabilization or preferential cleavage of target RNAs (Baek et al., 2008; Ha and Kim, 2014). During the last decade, substantial knowledge has accumulated regarding the biogenesis of microRNAs, their molecular mechanisms and functional roles in a variety of cellular contexts. Altered expression of certain microRNA molecules suggests that they could have a crucial regulatory role in disorders. Increasing evidence points to inflammation as a chief mediator of PD

with inflammatory response mechanisms, involving microglia and leukocytes, activated following loss of dopaminergic neurons (Rocha et al., 2015). The free radical nitric oxide (NO) plays a key role in the pathogenesis of inflammation. Under normal physiological conditions, NO has an anti-inflammatory effect, but is considered a pro-inflammatory mediator due to overproduction in abnormal situations (Sharma et al., 2007). The NO synthases (NOS) family synthesizes NO in a two-step reaction involving oxygen and many cofactors. Among the NOS isoforms (neuronal, endothelial, and inducible: nNOS, eNOs, iNOS, respectively), the nNOS is the most implicated in a wide range of functions and pathologies in the CNS. In the CNS, nNOS is located inside the postsynaptic membrane and is physically bound to N-methyl-D-aspartate (NMDA)-type glutamate receptors. Under physiological conditions, mild activation of synaptic NMDARs allows influx of Ca^{2+}, which leads to nNOS catalytic activation (Maccallini and Amoroso, 2016). By contrast, hyperactivation of extra-synaptic NMDARs can lead to an abnormal Ca^{2+} influx into the postsynaptic neuron, with a subsequent overstimulation of nNOS and excessive NO production. This leads to generation of reactive oxygen and nitrogen species that cause DNA and lipid damage (Heinrich et al., 2013; Maccallini et al., 2016). Consequently, neurotransmission is impaired due to mitochondrial dysfunction and synaptic damage. NO also induces apoptosis (Cao et al., 2005). Several microRNAs (miR-939, miR-26a) have been identified to bind with the human iNOS 3'-UTR and exert a translational blockade of human iNOS synthesis (Guo and Geller, 2014). Also, overexpression of microRNA-155 decreased, whereas inhibition of microRNA-155 increased, eNOS expression and NO production in human umbilical vein endothelial cells (Sun et al., 2012).

Oxidative stress is recognized as one of the main causes of PD, and excessive reactive oxygen species (ROS) can lead to dopaminergic neuron vulnerability and eventual death. Several studies have demonstrated that microRNAs can regulate oxidative stress in *in vitro* and *in vivo* animal models of PD. Relevant microRNAs involved in regulating oxidative stress can prevent ROS-mediated damage to dopaminergic neurons, suggesting that specific microRNAs may be putative targets for novel therapeutic strategies in PD (Xie and Chen, 2016). Impairment of mitochondrial function resulting in cellular damage is also linked to aging and neurodegeneration and evidence suggests it plays a central role in the pathogenesis of PD (Winklhofer and Haass, 2010). Glutamatergic transmission and inflammatory response mechanisms are altered in striatal neurons following dopaminergic denervation (Gardoni and Bellone, 2015; Kim et al., 2015). Despite extensive research, the molecular mechanisms mediating the changes in striatal neurons following dopaminergic denervation are still unclear. Understanding the mechanisms underlying this process is important for gaining new insights into the pathogenesis of PD. A recent study suggests that the age-related decline of Dicer enzyme combined with increased cellular stress in dopaminergic neurons may compromise microRNA biosynthesis thus contributing to neurodegeneration in PD (Chmielarz et al., 2017).

Circulating microRNAs have been proposed as diagnostic biomarkers for PD and would enable detection at the earliest stages of the disease for therapy to be implemented to delay the onset or minimize the changes in the later stages of the disease. However, other organs may contribute to microRNAs in the blood so that the circulating levels may not accurately reflect the levels of specific microRNAs in the diseased brain itself (Sierzega et al., 2017). We have searched the PubMed database for studies on microRNA expression in brain tissue of patients with PD and animal models of PD and their involvement in the pathophysiology of the disease, and which might serve as therapeutic targets using microRNA mimics or antagomirs. The studies retrieved in the literature search covered the period 2007–2017.

Neuropathology/Braak Staging and Animal Models of PD

The diagnosis of PD is still largely made on clinical grounds by four cardinal signs (tremor, bradykinesia, rigidity, and postural instability) as there is no definitive laboratory test to confirm the diagnosis during life, apart from gene testing in a reduced number of cases. Non-motor symptoms may predate diagnosis by several years and a schematic has been proposed depicting normal aging and PD-related nigral cell loss over time including the time at which diagnosis typically occurs (Noyce et al., 2016). Pre-symptomatic markers of PD may include olfactory loss, depression, rapid eye movement (REM) sleep disorder, and constipation (Schapira et al., 2017). Most reviews of PD indicate that motor signs first appear when approximately 50% of substantia nigra dopaminergic neurons are lost (Marsden, 1990; Ross et al., 2004). A regression analysis of neuron counts *versus* duration of PD indicated that the number of neurons lost at the time of symptom onset was 31%, adjusted for age (Fearnley and Lees, 1991). At the time of 1 year post diagnosis, patients with PD may retain up to 90% of their substantia nigra dopamine neurons and 50% of their striatal dopaminergic innervation (Kordower et al., 2013).

Based on autopsy findings in patients with PD, Braak et al. (2003) reported that the intraneuronal formation of Lewy bodies and Lewy neurites has a topographically predictable progression. Accordingly Braak staging was created based on the presence of Lewy bodies and Lewy neurites. The pre-symptomatic phase usually falls within Stages 1, 2 and 3, while the symptomatic phase falls into Stages 3, 4, 5 and 6. Stage 1 (medulla oblongata): lesions initially occur in the dorsal glossopharyngeal/vagal motor nucleus and frequently in the anterior olfactory nucleus. There may also be involvement of intermediate reticular zone. Stage 2 (medulla oblongata and pontine tegmentum): this includes the pathology of stage 1 together with lesions in the caudal raphe nuclei, gigantocellular reticular nucleus, and coeruleus-subcoeruleus complex. Stage 3 (midbrain): pathology of stage 2 plus midbrain lesions, particularly in the pars compacta of the substantia nigra. Stage 4 (basal prosencephalon and mesocortex): pathology of stage 3 with lesion at prosencephalon; cortical involvement is confined to the temporal mesocortex (transentorhinal region) and allcortex (CA2-plexus)-the neocortex is unaffected. Stage 5 (neocortex): pathology of stage 4 plus lesions in higher order sensory association areas of the neocortex and prefrontal neocortex. Stage 6 (neocortex): pathology of stage 5 plus lesions in first order sensory association areas of the neocortex and

premotor areas, occasionally mild changes in primary sensory areas and the primary motor field.

Neurotoxic and genetic animal models have been used to produce PD-related pathology and symptomatology (Blesa et al., 2012; Jackson-Lewis et al., 2012). Neurotoxin-based models produced by 6-hydroxydopamine (6-OHDA) and 1-methyl-1,2,3,6-tetrahydropyridine (MPTP) administration are the most widely used toxic models. Mice, rats, cats, dogs, and monkeys are all sensitive to 6-OHDA. Although similar in structure to dopamine, the presence of an additional hydroxyl group makes it toxic to dopaminergic neurons. This compound does not cross the blood-brain barrier (BBB), which necessitates its direct injection into the substantia nigra, medial forebrain bundle, or striatum. The most common use of 6-OHDA is via unilateral injection into the medial forebrain bundle or striatum. Injection of 6-OHDA into the substantia nigra kills approximately 60% of the tyrosine hydrolase (TH)-containing neurons in this area of the rodent brain with subsequent loss of TH-positive terminals in the striatum. The extent of the lesion depends on the amount of 6-OHDA injected, the site of injection, and the species used. This model does not mimic all the clinical features of PD. Dopamine depletion, nigral dopamine cell loss, and neurobehavioral deficits have been successfully achieved using this model, but it does not seem to affect other brain regions such as olfactory structures, lower brain stem areas, or locus coeruleus. Although 6-OHDA does not produce or induce proteinaceous aggregates or Lewy-like inclusions like those seen in PD, it has been reported that 6-OHDA does interact with α-synuclein (Blandini et al., 2008). 6-OHDA is frequently used as a unilateral injection because bilateral injection of this compound into the striatum produces severe adipsia, aphagia, and death (Ungerstedt, 1971).

MPTP represents the most important and most frequently used parkinsonian toxin applied in animal studies. It was shown to replicate almost all the hallmarks of PD including oxidative stress, ROS, energy failure, and inflammation. MPTP is highly lipophilic and rapidly crosses the BBB after systemic administration. Upon entering the brain, MPTP enters astrocytes and is metabolized into 1-methyl-4-phenylpyridinium (MPP+), its active metabolite which is a positively charged molecule, by monoamine oxidase-B. Once released from the astrocytes into the extracellular space *via* the OCT-3 transporter, MPP+ is taken up into the neuron by the dopamine transporter (DAT) and can be stored in vesicles. Inside the neuron, MPP+ is able to inhibit complex 1 of the mitochondrial electron transport chain, resulting in the release of ROS as well as decreased ATP production. MPP+ stored in vesicles is thought to expel dopamine into the extracellular space where it can be metabolized and subjected to superoxide and hydroxyl radical attack. MPTP is used mainly in nonhuman primates and mice, but has also been used in many other species such as dogs and cats. The MPTP mouse model is employed to study pathological effects of PD, while the MPTP monkey model is used mainly to study behavioral and symptomatic components of PD. The data generated by mouse models have led to a better understanding of molecular mechanisms involved in PD. .

In recent years a new generation of animal models of PD based on ectopic expression, overexpression, or intracerebral injection of α-synuclein have emerged (Visanji et al., 2016). Viral vector-mediated α-synuclein overexpression has been employed in rodents and nonhuman primates. Adeno-associated virus (AAV) vectors demonstrate high, maintained delivery of α-synuclein, with Lewy-like pathology, overt dopaminergic degeneration, and a parkinsonian behavioral phenotype in rodents (Koprich et al., 2010, 2011). These models develop inclusions of aggregated α-synuclein and/or α-synuclein-mediated neuronal cell loss replicating pathological hallmarks of PD and contributing to advances in the understanding of pathogenic mechanisms underpinning PD. Ip et al. (2017) showed that human mutated AAV1/2-A53T α-synuclein injected wild type-mice had widespread nigral and striatal expression of vector-delivered A53T α-synuclein. At 10 weeks, in AAV1/2-A53T α-synuclein mice there was a 33% reduction in TH$^+$ dopaminergic nigral neurons, 29% deficit in striatal DAT binding, and 38% reduction in dopamine level. The mouse model has certain advantages, especially it being amenable to genetic manipulation. Transgenic mice expressing α-synuclein have been generated to try to model PD to study α-synuclein pathobiology and investigate novel therapeutics (Magen and Chesselet, 2010; Koprich et al., 2017).

Accumulating evidence indicates that L-type calcium channels are involved in brain diseases such as PD (Ortner and Striessnig, 2016) and contribute to basal metabolic stress in substantia nigra dopaminergic neurons (Sulzer and Surmeier, 2013). Cav1.2 and Cav1.3 L-type calcium channels are expressed in the substantia nigra neurons (Ortner and Striessnig, 2016). They contribute to somatodendritic Ca^{2+} oscillations during autonomous pacemaking or bursting in these cells (Guzman et al., 2009). It is considered that this constant Ca^{2+} load contributes to the vulnerability of substantia nigra neurons to degeneration in PD by enhancing mitochondrial oxidative stress (Guzman et al., 2010). Epidemiological studies show that dihydropyridines, which are antagonists of these channels, reduce the observed risk of PD (Ritz et al., 2010). This finding is surprising given the relatively low affinity of dihydropyridines for the subtype of L-type calcium channel responsible for most of the calcium entry in striatonigral dopaminergic neurons, which is one with a Cav1.3 pore-forming subunit (Sinnegger-Brauns et al., 2009; Surmeier et al., 2011). Mice in which the gene for the Cav1.3 pore-forming subunit was deleted (Cav 1.3 knockout mice) and with impaired voltage-gated Ca^{2+} channel activity have been used to study PD.

Human studies

Thirteen studies were found and mostly comprised both male and female patients with mean ages ranging from 68 to 80 years (**Table 1**). Most of the studies indicated that the patients had sporadic PD. The three largest studies were performed on postmortem brain tissue samples from 22 patients (10 male/12 female, mean age 74 years; Tatura et al., 2016), 23 patients (5 male/2 female, Braak stages 1–3, mean age 68 years; 10 male/6 female, Braak stages 4–5, mean age 75 years; Miñones-Moyano et al., 2011) and 25 patients (4 male/2 female, Braak stages 1–2, mean age 79 years; 7 male/6 female, Braak stages 3–4, mean age 72 years; 4 male/2 female, Braak stage 5, mean age 80 years; Villar-Menendez et al., 2014). In the other studies,

brain tissues samples were examined from PD patients varying in number from 3 to 20, and although the Braak stages had not been included in most, neuropathological diagnoses of PD had been made according to recognized criteria such as the presence of Lewy bodies and neuronal loss in the substantia nigra. The mean postmortem interval (PMI) for collecting brain tissue samples ranged from 5 to 46 hours.

MicroRNAs upregulated in PD anterior cingulate gyri samples analyzed by RT-PCR were miR-199b, -544a, -488, -221, -144 and those downregulated were miR-7, -145, -543 (Tatura et al., 2016). An analysis of PD putamen samples by NanoString nCounter microRNA assay revealed 6 microRNAs were upregulated, miR-3195, -204-5p, -485-3p, -221-3p, -95, 425-5p, and 7 were downregulated, miR-155-5p, -219-2-3p, -3200-3p, -423-5p, -4421, -421, -382-5p (Nair and Ge, 2016). Using RT-PCR, miR-34b was downregulated in putamen samples of PD patients at Braak stages 1–2 and stages 4–5 (Villar-Menendez et al., 2014). Microarray analysis of PD substantia nigra samples revealed that one microRNA was upregulated, miR-548-d, while 10 were downregulated, miR-198, -485-5p, -339-5p, -208b, -135b, -299-5p, -330-5p, -542-3p, -379, -337-5p. Expression levels of miR-198, -548d, -135b were validated with individual TaqMan assays (Cardo et al., 2014). Also in PD substantia nigra samples, 6 microRNAs were upregulated, miR-21*, -224, -373*, -26b, -106a, -301b, and with similar but milder changes in amygdala samples with a significant upregulation of miR-224 and miR-373* (Alvarez-Erviti et al., 2013). By microarray analysis, 4 microRNAs were upregulated, miR-200b*, -200a*, -195*, -424*, and -7 microRNAs were downregulated, miR-200a, -199a-3p, -148a, -451, -144, -429, -190, in PD frontal cortex samples (Thomas et al., 2012). A significant downregulation of miR-34b and miR-34c occurred in PD amygdala, substantia nigra, and frontal cortex samples (Miñones-Moyano et al., 2011). Interestingly, a significant downregulation was also found in both miR-34b and miR-34c in the amygdala, but not in the frontal cortex, of PD pre-motor cases (Miñones-Moyano et al., 2011). By RT-PCR, miR-133b was downregulated in PD midbrain samples (Kim et al., 2007).

Two of the studies had included PD patients with dementia (PDD) (Cho et al., 2013; Hoss et al., 2016). By RT-PCR, miR-205 was downregulated in PD frontal cortex, and there was no significant difference in the expression level between PD patients and PDD patients. Downregulation of miR-205 also occurred in the PD striatum (Cho et al., 2013). By microRNA sequence analysis, the levels of 64 microRNAs were downregulated whereas the levels of 61 microRNAs were upregulated in PD prefrontal cortex compared to controls, and a set of 29 microRNAs classified PD from control brain (93.9% specificity, 96.6% sensitivity). 36 microRNAs classified PDD from PD without dementia (PDN) (88.9% specificity, 81.2% sensitivity). For the majority of differentially expressed microRNAs in PD, PDD samples exhibited larger differences than PDN for the same microRNAs (Hoss et al., 2016). Among the downregulated microRNAs in PD brains were let-7i-3p/5p, miR-184, -1224, -127-5p, and among the upregulated microRNAs was miR-16-5p (Hoss et al., 2016).

In addition, three studies were made on dopaminergic neurons in PD brain tissue samples (Choi et al., 2014; Schlaudraff et al., 2014; Briggs et al., 2015). A widespread expression of miR-7 was shown in dopaminergic neurons in PD substantia nigra (Choi et al., 2014). Also there was no difference in miR-133 level in dopaminergic neurons in PD substantia nigra compared to controls (Schlaudraff et al., 2014). In dopaminergic neurons collected from PD brain tissue, 8 of the 12 upregulated microRNAs were also upregulated in males, miR-106a, -135a, -148a, -223, -26a, -28-5p, -335, -92a, while 3 were upregulated in females, let-7b, miR-106a, -95 (Briggs et al., 2015).

Animal studies
Twelve studies in mice were found and males had been used where the gender was specified. Of these studies, 8 had used the MPTP model of PD, 2 the 6-OHDA model, 1 the α-synuclein overexpression model, and 1 the L-type calcium channel Cav1.3 knockout mouse model. The ages of the mice ranged from 6 weeks to 6 months (**Table 2**).

MPTP model studies
Mice received injection(s) of MPTP 20 mg/kg or 30 mg/kg over a period ranging from 1 to 21 days (for mice aged 6–12 weeks) or 5 weeks (for mice aged 4–5 months), and sacrificed at chosen time points after the last MPTP injection (if several injections were given). Many of the studies identified downregulation of specific microRNAs in brain tissue collected from these animals. Expression of miR-7 in the midbrain of MPTP-treated mice was downregulated compared to controls (Junn et al., 2009; Zhou et al., 2016). Similarly, following MPTP administration, miR-124 was downregulated in the ventral midbrain (Wang et al., 2016) and substantia nigra (Kanagaraj et al., 2014). Also, downregulation of miR-135a-5p was found in the brain tissue of mice after administering MPTP (Liu et al., 2016). By contrast, Su et al. (Su et al., 2016) found that miR-21 was upregulated in midbrain of MPTP-treated mice compared to controls. Xiong et al. (Xiong et al., 2014) showed that miR-494 was expressed highly in the substantia nigra of MPTP-treated mice and that overexpression of miR-494, induced by injecting lentivirus containing miR-494 into the substantia nigra, exacerbated MPTP-induced neurodegeneration, with a loss of dopaminergic neurons.

Expression of miR-7116-5p was downregulated while that of miR-125-5p was upregulated in microglia from the ventral midbrain of MPTP-treated mice (He et al., 2017). Also, miR-124 expression was downregulated in substantia nigra dopaminergic neurons following MPTP administration (Wang et al., 2016).

6-OHDA model studies
Mice received a unilateral injection of 6-OHDA 3.75 μg in the medial forebrain bundle (Rivetti di Val Cervo et al., 2017) or 10 μg in the striatum (Saraiva et al., 2016). Treatment of 6-OHDA-treated mice with three transcription factors, NEUROD1, ASCL1 and LMX1A, and the microRNA miR-218, collectively designated NeAL218, reprogrammed astrocytes *in vivo* into induced dopamine neurons (Rivetti di Val Cervo et al., 2017). MiR-124 nanoparticles (NPs) in mice, receiving a double injection to deliver 6-OHDA into the right striatum and miR-124 NPs into the right lateral ventricle, promoted an increase in migrating neuroblasts and enhanced brain repair (Saraiva et al., 2016).

α-Synuclein overexpression model study
Expression of miR-155 in the substantia nigra was upregulat-

ed in mice receiving a unilateral injection of AAV containing α-synuclein into the substantia nigra (Thome et al., 2016).

L-type calcium channel Cav1.3 knockout mouse model study
Expression of miR-204-5p and miR-143-3p was upregulated in the hippocampus of Cav 1.3$^{-/-}$ knockout mice compared to controls (Gstir et al., 2014).

MicroRNAs as Therapeutic Targets for PD
Dysregulated microRNAs in PD brain tissue samples
The human studies have identified a large number of microRNAs whose levels were dysregulated in PD brain tissue samples (**Table 1**). Included among those having downregulated expression were: miR-7, -145, -543 (cingulate gyri); miR-155-5p, -219-2-3p, -3200-3p, -423-5p, -4421, -421, -382-5p, -34b (putamen); miR-198, -485-5p, -339-5p, -208b, -135b, -299-5p, -330-5p, -542-3p, -379, -337-5p, -34b, -34c (substantia nigra); miR- 200a, -199a-3p, -148a, -451, -144, -429, -190, -34b, -34c -205 (frontal cortex); miR-34b, -34c (amygdala); miR-133b (midbrain); miR-205 (striatum); let-7i-3p/5p, miR-184, -1224, -127-5p (prefrontal cortex). Those with upregulated expression were: miR-199b, -544a, -488, -221, -144 (cingulate gyri); miR-3195, -204-5p, -485-3p, -221-3p, -95, 425-5p (putamen); miR-548-d, -21*, -224, -373*, -26b, -106a, -301b (substantia nigra); miR-224, -373* (amygdala); miR-200b*, -200a*, -195*, 424* (frontal cortex); miR-16-5p (prefrontal cortex). It would seem that different regions of the PD brain exhibit differently altered microRNA profiles (**Figure 1**), and this may reflect differences in the numbers and functional states of specific cell types present.

Several dysregulated microRNAs may be potential therapeutic targets, but none of the studies had examined the effect of modifying the expression levels of chosen microRNAs in the PD brain. Choi et al. (2014) had suggested that the overexpression of miR-205 may provide an applicable therapeutic strategy to suppress the abnormal upregulation of LRRK2 protein in PD brains. Overexpression could be achieved using a miR-205 agomir or mimic. Also miR-7 protected cells from MPP(+)-induced toxicity in human dopaminergic SH-SY5Y cells (Choi et al., 2014) and the use of a miR-7 agomir may improve PD brain pathophysiology. *In vitro* testing using agomirs to downregulated microRNAs or antagomirs to upregulated microRNAs may provide a way of identifying possible microRNAs targets to protect dopaminergic cells exposed to neurotoxins (MPP+ or 6-OHDA).

The animal studies also identified several microRNAs whose levels were dysregulated in brain tissues of PD models (**Table 2**). Included among microRNAs with downregulated expression were: miR-7 (midbrain, ventral midbrain); miR-124 (midbrain, substantia nigra); miR-135a-5p. Those with upregulated expression were: miR-21 (midbrain); miR-494, -155 (substantia nigra); miR-204-5p, -143-5p (hippocampus). He et al. (2017) found that the level of miR-7116-5p was downregulated while that of miR-125b-5p was upregulated in microglia from the ventral midbrain of MPTP mice. In MPTP model, overexpression of miR-494 by injection of lenti-494 into the substantia nigra exacerbated MPTP-induced neurodegeneration. Reprogramming of striatal astrocytes into induced do-

paminergic neurons occurred on injecting lenti-NeAl218 into the dorsal striatum of 6-OHDA model (Rivetti di Val Cervo et al., 2017). Injection of miR-124 nanoparticles into the lateral ventricle of 6-OHDA model enhanced brain repair (Saraiva et al., 2016). None of the studies reviewed had used agomirs or antagomirs to reverse the levels of downregulated or upregulated microRNAs, respectively, in *in vivo* mouse models of PD or in *in vitro* with isolated dopaminergic cells.

Downstream targets of dysregulated microRNAs in brain tissue samples
Downstream targets of several important microRNAs have been indicated in the PD studies reviewed. For example, downregulated *TNFSF13B* (TNF superfamily member 13b) is a predicted target of upregulated miR-425-5p and *LTA* (lymphotoxin alpha) and *SLC5A3* (soluble carrier family 5 sodium/myo-inositol cotransporter member 3) are predicted targets of upregulated miR-485-3p. The upregulated *PSMB2* (proteasome subunit, beta type 2) and *GSR* (glutathione reductase) are predicted targets of downregulated miR-423-5p and miR-219-3p, respectively (Nair and Ge, 2016). *LRRK2* (leucine-rich repeat kinase 2) is an experimentally validated target of downregulated miR-1224, and *GBA* (glucocerebrosidase) is a target of downregulated miR-127-5p and upregulated miR-16-5p (Hoss et al., 2016). Downregulated *LAMP-2A* (lysosome-associated membrane protein 2) and *HSC* (hematopoietic stem cell) are the predicted targets of upregulated miR-26b, -106a, -301b (Alvarez-Erviti et al., 2013). Also the 3'-UTR of transcription factor Ptx3 was identified as a potential target of downregulated miR-133b (Kim et al., 2007). Upregulated miR-21 in PD directly targeted the 3'-UTR of LAMP2A (lysosome-associated membrane protein 2A) (Su et al., 2016), while downregulated miR-135a-5p targeted the 3'-UTR of ROCK2 (rho-associated protein kinase 2) (Liu et al., 2016). Upregulated miR-204-5p and miR-143-3p were predicted to target the 3'-UTR of several ion channel mRNAs (Gstir et al., 2014). Also, the 3'-UTR of adenosine A_{2A} receptor ($A_{2A}R$) is a predicted target for downregulated miR-34b in PD (Villar-Menendez et al., 2014). Interestingly, dysregulated microRNA and target gene network related to PD may be gender-specific (Briggs et al., 2015).

Possible biological implications of some important dysregulated microRNAs in brain tissue samples
A single microRNA can regulate the expression of hundreds of target genes, so alterations in a panel of microRNAs could greatly affect the pathophysiology and outcome of PD. Use of *in vivo* animal models of PD together with *in vitro* studies using dopaminergic cells, neural progenitor cells or primary neurons exposed to MPP+ provide a means of testing specific microRNAs for protective or adverse effects. For instance, miR-7 was shown to protect dopaminergic neurons from MPP(+)-induced toxicity (Choi et al., 2014), and suppress NLRP3 inflammasone-mediated neuroinflammation in MPTP mouse model (Zhou et al., 2016). Upregulation of miR-205 may provide an applicable therapeutic strategy to suppress the abnormal upregulation of LRRK2 protein in PD brains (Cho et al., 2013). This could be achieved using lenti- or adeno-as-

Table 1 MicroRNAs in human patients with Parkinson's disease (PD).

Reference	No. of patients, gender, ages, tissue samples	Comparison	Changes in miRNAs in PD patients	Functional outcomes	Conclusion
Tatura et al. (2016)	22 Caucasian PD patients 10 male (M)/12 female (F), 73.9 ± 6.9 years of age, postmortem interval (PMI) 30.6 ± 17.4 hours, anterior cingulate gyri	10 Caucasian controls 4 M/6 F, 65.7 ± 10.9 years, PMI 29.4 ± 19.9 hours, anterior cingulate gyri	Of 744 microRNAs in gyri cinguli by microarray analysis, 43 were significantly upregulated (fold change ≥ 2.0) in brains of PD patients compared to controls. The same result was obtained when only same sex samples were compared. Five of the upregulated microRNAs had fold change > 4 (miR-591, -299-3p, -23a, -939, -10b). MicroRNAs downregulated in PD did not reach significance, but downregulation of two microRNAs (miR-623 and miR-1251) was almost significant. Upregulation of five of the microRNAs (miR-199b, -544a, -488, -221, -144) was confirmed by RT-PCR. However, upregulation of miR-17, -23a, -29b1, -30d, -424 could not be confirmed by RT-PCR, and three microRNAs (miR-7, -145, -543) found to be upregulated in microarray analysis were significantly downregulated in RT-PCR assays.	The 43 upregulated microRNAs were analyzed in databases for a potential role in the regulation of several genes implicated in the etiology of PD. From numerous genes implicated in monogenic forms of PD, $DJ-1$, $PARK2$, $PINK1$, $LRRK2$, $SNCA$, $HTRA2$ were selected. Three ($LRRK2$, $SNCA$, $PARK2$) of the six PD-related potential target genes studied were downregulated in patient brains. Expression of $SNCA$ appears to be modified by miR-144, -221, -488, and $PARK2$ by miR-199b, -221, -488. The observed reduced expression of $LRRK2$ could be mediated by miR-144. An additional 5 out of ten potential target genes tested were downregulated. These were $DRAM$ (DNA damage regulated autophagy modulator 1), predicted to be regulated by miR-144, EVC (Ellis Van Creveld protein) by miR-221, $ZNF440$ (Zinc finger protein 440) by miR-199b, $MTFMT$ (mitochondrial methionyl-tRNA formyltransferase) by miR-488 and $XIRP2$ (Xin actin binding repeat containing) possibly controlled by miR-544a.	5 microRNAs (miR-199b, -544a, -488, -221, -144) were identified that play a role in the etiology of Parkinson's disease likely by modifying expression of $SNCA$, $PARK2$, $LRRK2$, and additional genes required for normal cellular function.
Nair et al. (2016)	12 PD patients 6 M/6 F, 75.6 ± 8.4 years of age, PMI 13.4 ± 1.2 hours, 83.3% on L-dopa, putamen	12 controls 6 M/6 F, 74.1 ± 11.6 years, PMI 15.2 ± 1.0 hours, putamen	By NanoStringnCounter microRNA assay, the expression of approximately 250 microRNAs was detected in the human putamen tissues. Among them, a total of 13 microRNAs were dysregulated, of which 6 were significantly upregulated (miR-3195, -204-5p, -485-3p, -221-3p, -95, -425-5p) and 7 were downregulated (miR-155-5p, -219-2-3p, -3200-3p, -423-5p, -4421, -421, -382-5p) in PD patients *versus* controls. No significant difference in the expression of these microRNAs was found within or between the groups when tested for effect of age, gender, PMI. To confirm the results of microRNA profiling, RT-PCR was performed on 4 microRNAs (miR-3195, -204-5p, -155-5p, -219-2-3p) in all putamen samples. They were chosen as they had the highest differential expression in these samples. The four microRNAs were significantly different when compared with the controls.	To determine the abnormal inflammatory response mechanisms that exist in PD striatum, the expression of 134 genes implicated in inflammatory response and cell death was examined. The NanoStringnCounter mRNA assay was used to screen the transcripts involved in the inflammatory response pathway. Quantitation of the expression of the partially degraded mRNAs in PD and control putamen tissues showed that transcripts of $TNFSF13B$ (TNF superfamily, member 13), $ATF4$ (activating transcription factor 4), LTA (lymphotoxin alpha), $HMOX1$ (heme oxygenase-1), $SLC5A3$ (solute carrier family 5 sodium/myo-inositol cotransporter, member 3), and OSM (oncostatin M) were significantly downregulated, whereas $PSMB2$ (proteasome subunit, beta type 2), $CCL5$ (chemokine C-C motif ligand 5), GSR (glutathione reductase), and TXN (thioredoxin) were significantly upregulated in PD putamen. Downregulated $TNFSF13B$ is a predicted target of upregulated miR-425-5p and LTA and $SLC5A3$ are predicted targets of miR-485-3p. The upregulated $PSMB2$ and GSR are predicted targets of downregulated miR-423-5p and miR-219-3p, respectively. The downregulated genes $ATF4$ and $HMOX1$ are experimentally validated targets of the upregulated miR-135b-5p (Xue et al., 2013).	The expression of differentially regulated microRNAs had a negative correlation with the expression of genes implicated in the inflammatory response pathway in PD striatum.
Hoss et al. (2016)	29 sporadic PD patients 29 M, 11 with dementia (PDD) and 18 with no evidence of dementia (PDN). For PDD, 79.9 ± 9.0 years of age, disease duration 9.2 ± 6.7 years, PMI 9.9 ± 10.9 hours; for PDN, 76.1 ± 8.9 years, disease duration 11.5 ± 6.4 years, PMI 11.9 ± 9.2 hours, prefrontal cortex	33 controls 33 M, 68.1 ± 14.8 years, PMI 15.0 ± 8.7 hours, prefrontal cortex	By microRNA sequence analysis, 125 microRNAs were significantly altered in PD compared to controls after adjusting for age at death. The levels of 64 microRNAs were downregulated whereas the levels of 61 microRNAs were upregulated in PD relative to controls. A set of 29 microRNAs classified PD from control brain (93.9% specificity, 96.6% sensitivity). Among PD brains, 36 microRNAs classified PDD from PDN (88.9% specificity, 81.2 % sensitivity). In the majority of differentially expressed microRNAs in PD, PDD samples exhibited larger differences than PDN as compared to controls for the same microRNAs.	Several miRNAs that were altered in PD brain may interact with PD-related genes. Monogenic forms of PD include mutations within the α-synuclein gene ($SNCA$), leucine-rich repeat kinase 2 ($LRRK2$), one of the most common causes of familial PD, and glucocerebrosidase (GBA). While there were no alterations of $SNCA$-targeting miRNAs, miR-7 and miR-153, two microRNAs shown to be regulated by $LRRK2$ (let-7i-3p/5p and miR-184) and one microRNA experimentally shown to target $LRRK2$ expression (miR-1224) were downregulated in PD. Glucocerebrosidase (GBA) deficiency is associated with PD. MiR-127-5p, which has been shown to reduce GBA activity, was downregulated in PD brains, and miR-16-5p which has been shown to correspond to enhanced GBA protein levels was upregulated in PD brains.	Based on prefrontal cortex microRNA levels, PD brains were accurately classified from non-disease brains. The PDD microRNA profile exhibited a more severe pattern of alteration among those differentially expressed in PD.

Table 1 Continued

Reference	No. of patients, gender, ages, tissue samples	Comparison	Changes in miRNAs in PD patients	Functional outcomes	Conclusion
Briggs et al. (2015)	8 sporadic PD patients 5 M/3 F, ages N/A, PMI N/A, frozen brain tissue with about 300 dopaminergic (DA) neurons per sample collected by laser microdissection	8 controls 5 M/3 F matched for age and PMI with PD patients, frozen brain tissue with about 300 dopaminergic (DA) neurons per sample collected by laser microdissection	Using Megaplex TaqMan arrays and PCR, microRNA profiles were determined for all samples and males or females separately. A total of 159 microRNAs had Ct values < 35, with 109 being upregulated and 50 being downregulated. DA neurons from PD patients had dysregulated microRNA expression profiles with patterns of microRNA changes showing a trend of more upregulation in the male group and more downregulation in the female group.	Ingenuity Platform Analysis (IPA) was used to identify up- and down-regulated pathways or target genes. Dysregulated cellular pathways in PD DA neurons were, among others, related to apoptosis, disruption of filaments, cell proliferation, cell viability, and survival. These analyses identified 47 gene-targets of upstream regulators. When the upregulated microRNAs were correlated with the 47 targets of upstream regulators, 52 microRNAs were associated with 17 gene targets. From the 50 downregulated microRNAs, 4 targets of upstream regulators were identified that correlated with 8 microRNAs, from which 2 were significantly downregulated in PD and were associated with 2 target genes. Altogether a network of 14 significantly dysregulated microRNAs in PD DA neurons was correlated with 16 PD-associated target genes. As for gender differences, 8 of the 12 upregulated miRNAs with P values < 0.05 were also upregulated in males (miR-106a, -135a, -148a, -223, -26a, -28-5p, -335, and -92a), while 3 were upregulated in females (let-7b, miR-106a, and -95). Accordingly, 10 of the downregulated target genes (IRS2, STXBP1, TFRC, FHL1, VAV3, DDX17, HUWE1, CEBPB, LICAM, NEFL) were associated with males, 4 with females (ABCC5, AKAP12, IRS2, VAV3), and 1 (LMO3) with both.	Dysregulated microRNA and target-gene network related to PD may be gender-specific.
Villar-Menendez et al. (2014)	Total 25 PD patients: Braak 1–2 stages 4 M/2 F, 78.8 ± 12.1 years of age, PMI 6.0 ± 4.1 hours, Braak 3–4 stages 7 M/6 F, 72.3 ± 10.2 years, PMI 7.7 ± 6.6 hours, Braak 5 stage 4 M/2 F, 79.7 ± 6.2 years, PMI 10.5 ± 7.0 hours, putamen	26 controls 17 M/9 F, 56.9 ± 12.7 years, PMI 6.9 ± 4.2 hours, putamen	By RT-PCR, the expression levels of miR-34b and miR-34c were measured in Braak PD 1–2 stage samples and Braak PD 4–5 stages. MiR-34b was significantly reduced in the putamen of PD in early Braak stages (Braak 1–2) and in disease progression (Braak 4–5 stages) compared to control. MiR-34c was not significantly altered in Braak stages 1–2 or Braak stages 4–5 compared to control.	The 3'-UTR of adenosine A_{2A} receptor (A_{2A}R) contains a predicted target for miR-34b. In vitro studies revealed that endogenous A_{2A}R protein levels increased when miR-34b function was blocked using a specific anti-miR-34b. Increased A_{2A}R protein levels in plasma membrane extracts were found in the putamen of early PD stages (Braak 1–2 stages) compared to controls. The increase at early Braak stages was comparable to that observed at advanced Braak stages 4–5.	Increased striatal A_{2A}R level is an early event in PD pathology and is potentially regulated by miR-34b. A_{2A}R is a G-protein coupled receptor that stimulates adenylyl cyclase activity in the brain.
Schlaudraff et al. (2014)	5 PD sporadic patients Braak 2–5 stages 3 M/2 F, 78.2 ± 1.3 years of age, PMI 16.2 ± 3.2 hours, RNA integrity number (RIN) 7.3 ± 0.2, midbrain	8 controls 4 M/4 F, 69.0 ± 1.6 years, PMI 39.4 ± 13.4 hours, RIN 6.3 ± 0.1, midbrain	Donor age of PD brains was significantly higher compared with the control group. The relative amount of microRNA was also lower in PD (14.2 ± 1.6%) than in control brains (24.9 ± 1.8%). By RT-PCR, the miR-133b level tended to be lower in PD midbrain but not significantly different to controls. No difference in miR-133b level was detected in substantia nigra (SN) DA neurons between PD and controls.	In accordance with unaltered miR-133b levels, no changes were found in PIX3 and NURR1 levels in PD compared to controls, at both tissue and SN DA specific level. PIX3 and NURR1 expression was suggested to be regulated by miR-133b and important for the SN DA neuronal phenotype. Mathematical adjustment of cell-specific data for age and RIN effects of NURR1 expression suggested a downregulation of NURR1 in PD. In contrast to miR-133b, PITX3, and NURR1, mRNAs for tyrosinehydroxylase (TH), the rate limiting enzyme for dopamine synthesis, as well as for SNCA were dramatically increased in SN DA neurons from PD patients compared with controls. Also expression levels of the plasma membrane dopamine transporter (DAT), important for axonal and somatodendritic dopamine reuptake as well as for the vesicular monoamine transporter 2 (VMAT2), were significantly increased. Interestingly, increased expression of VMAT2 in PD is not preserved after adjustment for RIN and age effects. Only the most prominent elevation of TH in SN DA neurons from PD brains compared to controls was still detectable at the midbrain tissue level.	MiR-133b levels were unaltered in midbrain tissue and SN DA neurons from PD brains. Differences in gene expression changes were found between midbrain and SN DA neurons of PD brains. In addition to the dramatic reduction of DA neurons in midbrain tissue in PD (motor symptoms in PD manifest when approximately 70% of SN DA neurons are lost, Darnier et al., 1999), analysis at the tissue level is additionally confounded by altered numbers and functional states of non-neuronal cells such as microglia, astrocytes and local T-cells. Thus, cell-specificity is crucial when comparing changes in gene expression of SN in PD and control states.

Table 1 Continued

Reference	No. of patients, gender, ages, tissue samples	Comparison	Changes in miRNAs in PD patients	Functional outcomes	Conclusion
Choi et al. (2014)	PD patients, no. N/A, gender N/A, ages N/A, PMI N/A, brain sections		Fluorescence *in situ* hybridization (FISH) for miR-7, together with immunostaining for TH as a marker for dopaminergic neurons, revealed widespread expression of miR-7 in SN sections in TH-positive neurons.	MiR-7 protected cells from 1-methyl-4-phenylpyridinium (MPP+)-induced toxicity in dopaminergic SH-SY5Y cells, differentiated human neural progenitor ReNcell VM cells, and primary mouse neurons. RelA/p65, a component of nuclear factor-κB (NF-κB) was downregulated by miR-7. RelA is a target of miR-7 and is required for cell death following MPP+ exposure. RelA mediates MPP(+)-induced suppression of NF-κB activity, which is essential for MPP(+)-induced cell death.	FISH suggested miR-7 plays a physiological role in dopaminergic neurons. *In vitro* experiments showed the protective effect of miR-7 is exerted through relieving NF-κB suppression by reducing RelA expression.
Cardo et al. (2014)	8 PD patients 3 M/5 F, 77.4 ± 3.2 years of age, disease duration 4.3 ± 0.9 years, PMI 45.9 ± 8.5 hours, substantia nigra tissue samples previously studied for several PD-candidate genes and negative for mutations in *SNCA, PRKN, LRRK2* genes	4 controls 2 M/2 F, 69.0 ± 5.9 years, PMI 30.3 ± 4.6 hours, substantia nigra	By microarray analysis, the expression values of 733 microRNAs were compared and only 11 were significantly different between the PD patients and controls. Of these microRNAs, 10 were downregulated (miR-198, -485-5p, -339-5p, -208b, -135b, -299-5p, -330-5p, -542-3p, -379, -337-5p) and only one miR-548-d had a significantly higher expression in the patients. No microRNA was found in all the patients but none of the controls and *vice versa*. MicroRNAs previously related with PD in other studies (miR-34b/c, - 133, -433, -7, -184) were not significantly different between PD patients and controls. As part of the validation of the microarray assays, miR-198, -485-5p, -548d, -135b were tested with individual TaqMan assays. The microRNAs showed similar Ct values to those in the microarray assays for the 12 samples. Among these, miR-135b showed the most significant difference between PD patients and controls.	Among the 11 microRNAs that were significantly different between PD patients and controls, miR-339-5p, -198, -485-5p, -548d have been previously implicated in neurodegenerative disorders (Long et al., 2014).	A general downregulation of microRNAs was found in the substantia nigra of PD patients compared to controls. The expression of 11 microRNAs was significantly different between PD patients and control tissues, but none was present in one of the groups and absent in the other.
Cho et al. (2013)	8 sporadic PD patients, 3 M/5 F, 78.8 ± 3.3 years of age, PMI 18.4 ± 3.9 hours, frontal cerebral cortex. 12 sporadic PD patients with dementia (PDD), 9 M/3 F, 74.5 ± 1.9 years, PMI 18.7 ± 4.5 hours, frontal cerebral cortex,	10 controls, 5 M/5 F, 80.6 ± 1.7 years, PMI 19.6 ± 3.6 hours, frontal cerebral cortex	By RT-PCR, there was a significantly lower level of miR-205 expression in frontal cerebral cortex of PD patients (n = 15) compared to controls (n = 11), whereas no significant difference was found between PD patients and PDD patients. No significant differences in miR-181, -19, and -410 were found between PD and control brains. In addition to the frontal cortex, miR-205 was significantly downregulated in the striatum of sporadic PD patients (n = 5) compared to controls (n = 4). Levels of miR-410 expression showed no difference in the striatum of sporadic PD patients compared to controls.	The expression of leucine-rich repeat kinase 2 (*LRRK2*) protein was significantly upregulated in the frontal cortex of PD patients (n = 8) and PDD patients (n = 8) compared to controls (n = 7). Increased LRRK2 protein expression was observed in the brain of PD patients using two different LRRK2 antibodies. The levels of LRRK2 mRNA were not significantly different between PD, PDD and control groups, suggesting a potential post-transcriptional modification of LRRK2 protein expression in the sporadic PD brains.	Downregulation of miR-205 may contribute to the potential pathogenic elevation of LRRK2 protein in the brains of sporadic PD patients, while the overexpression of miR-205 may provide an applicable therapeutic strategy to suppress the abnormal upregulation of LRRK2 protein in PD brains.
Alvarez-Erviti et al. (2013)	6 PD patients, 5 M/1 F, 76.7 ± 1.4 years of age, PMI 4.8 ± 1.3 hours, substantia nigra and amygdala	5 controls, 2 M/3 F, 70.2 ± 3.1 years, PMI 4.8 ± 1.0 hours, substantia nigra and amygdala	The levels of the three microRNAs targeting *Lamp-2a* (miR-21*, -224, -373*) and the three microRNAs targeting *hsc70* (miR-26b, -106a, -301b) were significantly increased in PD substantia nigra relative to actin mRNA levels. These increases corresponded to a significant decrease in *Lamp-2a* (71%) and *hsc70* (78%) mRNA levels and a concomitant decrease in LAMP-2A (45%) and *hsc70* (51%) protein levels previously reported (Alvarez-Erviti et al., 2010). Similar but milder changes were found in PD amygdala where there was a significant increase in the two microRNAs targeting *Lamp-2a* (miR-224 and miR-373*) and a nonsignificant increase in the two microRNAs targeting *hsc70* (miR-26b and miR-106a). These were associated with a mild decrease in LAMP-2A (36%) and *hsc70* (32%) protein levels and a mild downregulation of *Lamp-2a* (30%) and *hsc70* (10%) mRNA levels. A significant decrease was detected in the α-synuclein mRNA levels in both the substantia nigra and amygdala samples from PD patients.	Eight microRNAs were identified that are predicted to regulate the chaperone-mediated autophagy (CMA) proteins LAMP-2a and hsc70 reported to be increased in PD brains.	6 and 2 of the microRNAs targeting *Lamp-2a* and *hsc70* were significantly increased in substantia nigra and amygdala respectively and corresponded to decreases in CMA proteins LAMP-2a and hsc70. It is suggested that decreased CMA caused by microRNA-induced downregulation of CMA proteins plays an important role in the α-synuclein pathology associated with PD. Modulation of CMA function in PD by microRNA silencing might represent a suitable target for drug intervention.

Table 1 Continued

Reference	No. of patients, gender, ages, tissue samples	Comparison	Changes in miRNAs in PD patients	Functional outcomes	Conclusion
Thomas et al. (2012)	8 PD patients, gender N/A, ages N/A, PMI N/A, frontal cortex	8 controls, gender N/A, ages N/A, PMI N/A, frontal cortex	By microarray analysis, 48 microRNAs were regulated with > 2-fold change in PD frontal cortex compared to controls. Of these, 11 microRNAs families were predicted that could interact with master metabolism and mitochondrial biogenesis transcription factor PGC-1α or its upstream regulators MEF2, FOXO1, ATF2, CREBx. 4 microRNAs were upregulated (miR-200b*, -200a*, -195*, 424*) and 7 microRNAs were downregulated (miR-200a, -199a-3p, -148a, -451, -144, -429, -190) in PD frontal cortex samples compared to controls.	In PD frontal cortex mitobiogenesis signaling relationships are maintained but downregulated, correlate with impaired mitochondrial NADH-driven electron flow and may arise from combinations of nitrosative/oxidative stresses, inflammatory cytokines, altered levels of mitobiogenesis gene-interacting microRNAs, or other unknown mechanisms. Impaired mitochondrial biogenesis contributes to depletion of functional mitochondria in cells exposed to chronic mitochondrial stress (Zhu et al., 2012).	Changes were found in microRNAs of PD brains that could alter PGC-1α expression, as well as downregulation of mitobiogenesis in human neurons by combinations of nitrosative/oxidative stresses, inflammatory cytokines. Stimulation of mitobiogenesis in PD may inhibit rostal disease progression and appearance of secondary symptoms referable to frontal cortex.
Miñones-Moyano et al. (2011)	PD patients, 5 M/2 F, Braak stages 1–3, 68.1 ± 6.2 years of age, PMI 6.4 ± 0.9 hours; 10 M/6 F Baak stages 4 and 5, 74.6 ± 2.8 years, PMI 8.0 ± 1.6 hours, amygdala, substantia nigra, frontal cortex, cerebellum	Total no. controls 17 M/11 F, 58.6 ± 3.0 years, PMI 5.9 ± 0.6 hours, amygdala, substantia nigra, frontal cortex, cerebellum	By microarray analysis, expression of microRNAs in amygdala of 11 PD patients was compared with that of 6 controls. 2 microRNAs (miR-637 and miR-34c-5p) were downregulated by > 40% in 9 out of the 11 PD samples compared to control group. RT-PCR confirmed significant downregulation of miR-34c in the amygdala of PD patients but not of miR-637. The expression of both miR-34b and miR-34c was evaluated in additional symptomatic PD and control amygdala samples. PD amygdala showed a significant decrease in the expression of miR-34b and miR-34c by ~55% and 65%, respectively, *versus* controls. The expression of miR-34b and miR-34c was also decreased in the substantia nigra of PD patients by 40% and 45%, respectively. A significant decrease (~55%) in both miR-34b and miR-34c was detected in the frontal cortex of PD patients. The cerebellum, a structure with virtually no lesions in PD, had a less robust and close to significance downregulation of miR-23b (~23%) and a significant downregulation of miR-34c (~43%). DJ-1 and Parkin expression was significantly reduced in PD amygdala, which presented strong miR-34b/c downregulation.	To assess whether miR-34b/c downregulation correlated with evolution of the disease, the expression of these microRNAs was evaluated in the amygdala and frontal cortex of PD patients at pre-motor stages (Braak stages 1–3). A significant decrease was found in the expression of both miR-34b and miR-34c of 35% and 45%, respectively, in the amygdala of PD pre-motor cases compared to controls. The decrease in miR-34b/c expression in the frontal cortex of PD pre-motor cases did not reach statistical significance. None of the patients having neuropathological changes of PD-related pathology Braak stages 1–3 received any treatment related to PD.	It was proposed that early deregulation of miR-34b/c in PD triggers downstream transcriptome alterations underlying mitochondrial dysfunction and oxidative stress, which ultimately compromise cell viability.
Kim et al. (2007)	3 PD patients, gender N/A, ages N/A, PMI N/A midbrain, cerebellum, cerebral cortex	3 controls, gender N/A, ages N/A, PMI N/A midbrain, cerebellum, cerebral cortex	By RT-PCR, expression analyses were performed for a panel of 224 microRNA precursors. Expression of one of these precursor microRNAs, miR-133b, was specifically enriched in midbrain of controls but not in PD midbrain.	The 3′-untranslated region (3′ UTR) of transcription factor Pitx3 was identified as a potential target of miR-133b activity.	It was proposed that miR-133b functions within a feedback loop as Pitx3 specifically induces transcription of miR-133b and Pitx3 is downregulated by miR-133b post-transcriptionally.

N/A: Not available.

Table 2 MicroRNAs in animal models of Parkinson's disease (PD)

Reference	No. of animals, gender, ages	Comparison	Changes in miRNAs in animals	Functional outcomes	Conclusion
MPTP mouse model					
He et al. (2017)	9 C57BL/6 (wild-type) mice, male, 8–10 weeks of age, injected i.p. with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) (20 mg/kg) every 2 hours for a total of 4 doses in 1 day. Animals were sacrificed at 1 day and 3 days after MPTP administration and brains removed. Microglia were isolated from the ventral midbrain. CD11b-Cre mice, male, no. N/A, 8–10 weeks of age, injected with LSL-GFP-miR-7116 adeno-associated virus (AAV) particles (1×10^9, 1 μL) was slowly injected into the substantia nigra at a rate of 0.1 μL/min.	8 wild-type mice, male, 8–10 weeks of age, injected i.p. with saline every 2 hours for a total of 4 doses in 1 day. Animals were sacrificed at 1 day and 3 days after saline administration and brains removed. Microglia were isolated from the ventral midbrain. CD11b-Cre mice, male, no. N/A, male, 8–10 weeks of age, injected with LSL-GFP-AAV (1×10^9, 1 μL) into the substantia nigra at a rate of 0.1 μL/min	By RT-PCR, the level of miR-7116-5p in microglia from the ventral midbrain of the mice at 1 day ($n = 4$) and 3 days ($n = 5$) after MPTP administration was significantly reduced compared to that in microglia from the ventral midbrain of the mice at 1 day ($n = 4$) and 3 days ($n = 4$) after being injected with saline. Also the level of miR-125b-5p in the microglia from the ventral midbrain of the mice was significantly increased at 1 day after MPTP administration, but not at 3 days, compared to that in microglia from the ventral midbrain of the mice injected with saline. 1-methyl-4-phenylpyridinium (MPP⁺) specifically potentiated tumor necrosis factor (TNF)-α production in microglia.	LSL-GFP-miR-7116 AAV was delivered to the ventral midbrain of CD11b-Cre recombinase transgenic mice to express miR-7116 specifically in microglia. The expression of TNF-α in ventral midbrain was greatly reduced in CD11b-Cre mice injected with LSL-GFP-miR-7116 AAV, compared with those injected with LSL-GFP AAV, 1 day after administration of MPTP, while the levels of IL-1β, IL-6, iNOS, and COX-2 were not changed between the two groups. At 3 days after administration of MPTP, the expression of these factors was largely reduced in LSL-GFP-miR-7116/CD11b-Cre mice, compared to that in LSL-GFP/CD11b-Cre mice. The LSL-GFP-miR-7116 AAV alone did not change the number of DANs in substantia nigra. However, the DAN loss in LSL-GFP-miR-7116 AAV-injected CD11b-Cre mice 7 days after MPTP was markedly prevented compared to that in LSL-GFP-AAV-injected CD11b-Cre mice.	Downregulation of miR-7116-5p in microglia initially potentiated the production of TNF-α after MPTP, which then amplified the production of other proinflammatory factors to contribute to DAN damage.
Zhou et al. (2016)	Wild type and A53T^tg/tg mice, no. N/A, male, 4–5 months of age. MPTP (20 mg/kg) in saline was injected s.c. followed by probenecid (250 mg/kg) in DMSO injected i.p. at 1 hour interval every 3.5 days over a period of 5 weeks. Animals sacrificed 1 week after the final injection and brains removed.	Wild type and A53T^tg/tg mice, male, 4–5 months of age injected with saline and probenecid	By RT-PCR, miR-7 levels were reduced by approximately 60% and 55% in the midbrain in the MPTP/p-treated mice and A53T^tg/tg mice, respectively.	Injection of miR-7 into wild type mice treated with subacute MPTP was performed to evaluate the protective effect of miR-7 on DANs. Injection of miR-7 mimics inhibited IBA-1⁺ microglial activation and rescued the loss of TH⁺ neurons in the substantia nigra of MPTP-treated mice ($n = 6$). MiR-7 mimics had no effect on the numbers of IBA-1⁺ and TH⁺ cells in mice without MPTP administration. Injection of miR-7 mimics into the striatum of A53T^tg/tg mice significantly downregulated midbrain NLRP3 inflammasone expression accompanied by inhibition of caspase-1 activation and reduction of IL-1β production while having no significant effect on TH⁺ cell number in A53T^tg/tg mice at basal state.	MiR-7 protects DANs against PD-like degeneration by suppressing NMLRP3 inflammasone-mediated neuroinflammation.
Wang et al. (2016)	36 C57BL/6 mice, male, 8–10 weeks of age, received one i.p. injection of MPTP-HCl (30 mg/kg) per day for 5 consecutive days. Mice were sacrificed at different time points after MPTP administration: 0 (immediately after the last MPTP injection), 1, 2, 4, 7, 21 days after the last MPTP injection ($n = 6$/group). The brains were removed and the ventral midbrain was dissected. For exogenous delivery of miR-124 in animal model, the right lateral ventricle was surgically implanted with a stereotactic catheter. After 1 week of recovery, mice received one treatment of agomir miR-124-3p (20 nM of ribonucleotide in 5 μL) through the catheter per day for 5 consecutive days. The treatment of agomir was performed 2 days prior to the injection of MPTP.	Control mice received saline injection only. Controls for exogenous delivery of miR-124 in animal model received agomir-negative control sequences injected into the right lateral ventricle.	By RT-PCR, the expression of miR-124 in the midbrain decreased after i.p. injection of MPTP. By in situ hybridization, miR-124 expression was downregulated in substantia nigra DANs.	The density of TH⁺ neurons was higher in the miR-124 agomir group than in the negative control group at 21 days after MPTP treatment. Loss of striatal dopamine was significantly less pronounced in the miR-124 agomir group compared to the negative group. The upregulation of Bim mRNA level and protein level induced by MPTP was reduced by miR-124 agomir compared to the negative control. There was no change in protein expression of Puma and Noxa in MPTP-treated mice. The pro-apoptotic effect of Bim is mediated through Bax mitochondrial translocation in the MPTP model of PD (Perier et al., 2007) and was reduced by upregulation of miR-124. Correspondingly the number of apoptotic cells in the substatia nigra was reduced in the miR-124 agomir group.	Upregulation of miR-124 could regulate apoptosis thus reducing the loss of DANs.
Su et al. (2016)	C57BL/6N mice, male, 10 weeks of age. Geniposide (GP) group mice ($n = 8$) were treated with 100 mg/kg GP daily by intragastric gavage for 21 days. MPTP group mice ($n = 8$) were injected i.p. with 20 mg/kg MPTP every 8 hour for 21 days to establish PD mouse model. MPTP + GP group mice ($n = 16$) were treated with MPTP for 1 hour and then given 100 mg/kg GP daily by intragastric gavage for 21 days. GP+ miR-21 group mice ($n = 16$) were given 100 mg/kg GP daily by intragastric gavage and intracerebroventricular injection of miR-21 agomir or negative control (NC) where the miR-21 agomir or NC had been diluted to 70 μM and 5.0 μL of it mixed with 12.5 μL lipofectamine 2000 and incubated at 25°C for 30 minutes. Intracerebroventricular injection was carried out 1 hour after the first treatment with GP. Mice were sacrificed and the midbrain removed. Immunostaining of ventral midbrain sections was used to count the number of TH⁺ neurons. In the GP+ miR-21 group, the mice were treated with MPTP, GP and miR-21 agomir.	8 mice were given 0.2 mL saline every 24 hours for 21 days	By RT-PCR, the level of miR-21 was significantly increased in MPTP group. MPTP significantly decreased the number of TH⁺ cells in the substantia nigra but GP significantly inhibited the decrease of ipsilateral TH⁺ cells caused by MPTP. However, after treatment with MPTP + GP + miR-21 agomir, ipsilateral TH⁺ cells were significantly downregulated again compared to that of MPTP + GP group.	GP inhibited the promotion effect of MPTP on miR-21 levels and miR-21 agomir markedly increased the level of miR-21 in midbrain. GP also was shown to increase the protein and mRNA expression of lysosome-associated membrane protein 2A (LAMP2A) and decreased the protein level of α-synuclein in the PD mouse model. MiR-21 upregulated the expression of α-synuclein by directly targeting 3′-UTR of LAMP2A.	GP shows neuro-protective properties by inhibiting α-synuclein expression in PD mice through the miR-21/LAMP2A axis.

Table 2 Continued

Reference	No. of animals, gender, ages	Comparison	Changes in miRNAs in animals	Functional outcomes	Conclusion
Liu et al. (2016)	10 C57BL/6J mice, male, 12 weeks of age. MPTP 20 mg/kg was injected s.c. daily from day 4 to day 8 and injected i.p. with saline daily from day 0 to day 12. Then mice were sacrificed for analyzing brain tissue.	10 mice injected s.c. with saline daily from day 4 to day 8 and injected i.p. with saline daily from day 0 to day 12	By RT-PCR, MPTP administration decreased miR-135a-5p levels in the brain tissue.	MiR-135a-5p targets the 3′-UTR of ROCK2 mRNA. Microglial ROCK2 is upregulated by MPTP induction and leads to phagocytosis of dopaminergic neurons. MPTP induces astrocyte activation in the mouse striatum.	The downregulation of miR-135a-5p in the mouse MPTP model of PD is associated with an upregulation in ROCK2 which leads to phagocytosis of dopaminergic neurons.
Xiong et al. (2014)	C57BL/6 mice, male, 6 weeks of age. Lentivirus containing miR-494 (lenti-miR-494, 2 µL for each side) was stereotactically injected into both sides of substantia nigra at a rate of 1 µL/min. Two weeks after lentivirus injection, mice were injected i.p. at 1 injection/day for 5 consecutive days with 30 mg/kg MPTP-HCL. Animals were sacrificed at 2, 4, 7, 14, 21 days, and substantia nigra analyzed.	Mice injected with empty lentivirus (lenti-NC)	Using RT-PCR, miR-494 was expressed highly in the substantia nigra of the MPTP mouse model. Then lentivirus-mediated gene transfer was used to overexpress miR-494 in the substantia nigra of mouse before MPTP administration.	MiR-494 expression in the substantia nigra was greater in mice stereotactically injected with lenti-miR-494 compared with the lenti-NC mice. With the increasing of miR-494, DJ-1 expression level was decreased in the substantia nigra after MPTP administration, whereas mRNA of DJ-1 remained unchanged, indicating that the regulation of miR-494 was limited to the translation level without affecting the mRNA stability. MiR-494 expression was inversely correlated with DJ-1 in mouse substantia nigra. MPTP administration induced moderate but not overt dopaminergic neurodegeneration in substantia nigra of lenti-NC-injected mice, and stereotactic injection with lent-miR-494 resulted in a significant decrease in TH-immunoreactive neurons in whole substantia nigra compared to the lenti-NC group.	In MPTP mouse model, overexpression of miR-494 negatively regulated DJ-1 levels and exacerbated MPTP-induced neurodegeneration, as shown by the loss of dopaminergic neurons.
Kanagaraj et al. (2014)	C57BL/6 mice, male, 8–10 weeks of age, were given 4 injections of MPTP-HCl at 2 hours intervals (total dosage 72 mg/kg). Animals were sacrificed at 1, 3, 5, 7, 10 days after the last MPTP injection. The substantia nigra was dissected bilaterally for analysis	Mice were injected with an equal volume of saline	By RT-PCR array, the expression of mIR-124 in the substantia nigra of mice treated with MPTP, isolated by laser capture microdissection, was decreased on day 5 post-treatment compared to saline-injected mice.	The expression of calpains 1 and 2 which is modulated by miR-124 was increased in the substantia nigra of MPTP-treated mice and in MN9D dopaminergic neurons treated with MPP iodide leading to increased expression of the p35 cleavage product, p25 and cyclin-dependent kinase 5 (cdk5). Calpain-p25-mediated increase in cdk5 expression leads to dopaminergic neuronal death. Overexpression of miR-124 after MPP iodide treatment on MN9D cells attenuated the calpain 1/p25/cdk5 proteins and improved cell survival.	Controlling the expression of miR-124 will aid in targeting miR-124 for better treatment strategies for PD.
Junn et al. (2009)	C57BL/6 mice, male, 12 weeks of age, received i.p. injections of MPTP-HCl (30mg/kg/day) for 5 consecutive days. Animals were sacrificed 14 days after the last injection and brains removed.	Mice received saline injections.	By RT-PCR, subchronic MPTP administration resulted in 50% decease in miR-7 expression in ventral midbrain.	The expression of miR-7 in neurons is 40-fold higher than in astrocytes. α-Synuclein was detected in neurons but not in astrocytes. This suggests that miR-7 negatively regulates α-synuclein expression in neurons	It is suggested the decrease in miR-7 expression is involved in degeneration of the nigrostriatal system in the MPTP mouse model, likely through up-regulation of α-synuclein expression.
6-OHDA mouse model					
Rivetti di Val Cervo et al. (2017)	*B6.Cg-Tg(GFAP-tTA)110Pop// mice, no. N/A, gender N/A, adult 2–6 months of age. For dopamine depletion in the right striatum, mice received a unilateral injection of 6-hydroxydopamine-HCl (6-OHDA–HCl) into the right medial forebrain bundle. Each mouse was injected with 1 µL (0.2 µL/min) of 6-OHDA 3.75 µg/µL in 0.02% ascorbic acid in saline. Mice were allowed to recover for 2 weeks. Dopamine-depleted GFAP-tTA mice were injected in the right striatum with high-titer lentiviruses or adeno-associated virus (AAV). Each mouse received one injection of 1.5 or 2 µL (0.1µL/min) into the right dorsal striatum. Using three distinct transcription factors, NEUROD1, ASCL1 and LMX1A, and the microRNA miR-218, collectively designated NeAL218, were tested to reprogram mouse astrocytes in vivo into induced dopamine neurons (IDANs)*	*B6.Cg-Tg(GFAP-tTA)110Pop// mice, weight-matched, unilaterally injected with 6-OHDA and treated with GFP*	Tet-regulated NeAL218 or GFP lentiviruses were injected into the ipsilateral striatum of transgenic mice in which the tetracycline transactivator is under the control of the gfap promoter. This genetic construct allows while the expression of transgenes exclusively in astrocytes, in the absence of doxycycline. Two weeks after NeAL218 injection, TH⁺ cells were identified at different stages of reprogramming including TH⁺;GFAP⁺ cells with mixed astrocyte-to-neuron morphology, as well as TH⁺;GFAP⁻ and TH⁺;SLC6A3⁺ cells with neuronal morphology. Newly generated iDANs were either DCX⁺ (doublecortin, a marker for early migratory neurons) or the mature neuronal marker RBFOX3⁺, showing that they mature at variable rates into neurons. Notably, iDANs were abundant by 15 weeks after 6-OHDA injection. Rotations induced by apomorphine decreased in mice treated with NeAL218, compared to GFP, 13 weeks after viral injections. Spontaneous circling behavior, which emerges as a consequence of the severe unilateral loss of striatal DA, was completely rescued by NeAL218, but not by GFP, 5 weeks after viral injection in mice unilaterally injected with 6-OHDA. Electrophysiological recordings revealed the capacity of iDANs to reliably generate action potentials.	In a mouse model of PD, NeAL218 alone reprograms adult striatal astrocytes into iDANs that are excitable and correct some aspects of motor behavior in vivo, including gait impairments.

Table 2 Continued

| Reference	No. of animals, gender, ages	Comparison	Changes in miRNAs in animals	Functional outcomes	Conclusion
Saraiva et al. (2016)	C57BL/6 mice, no. N/A, male, 10–12 weeks of age. MiR-124 nanoparticles were unilaterally injected into the lateral ventricle followed by 3 days of i.p. injections with BrdU every 12 hours. To unveil the effect of the NP formulation in a preclinical mouse model of PD, mice were subjected to a double stereotactic injection to deliver 6-OHDA (10 μg, in 0.02% ascorbic acid) into the right striatum and the miR-124 NPs into the right lateral ventricle. Mice were sacrificed at 4 weeks after stereotactic surgeries and the number of neuroblasts (doublecortin DCX⁺) and proliferating neuroblasts (DCX⁺/BrdU⁺) were counted in the supraventricular zone (SVZ) and in the granule cell layer (GCL) and glomerular layer (GL) of the olfactory bulb (OB)	Mice injected with saline both in the striatum and in the lateral ventricle ('healthy saline' group); mice injected with saline in the striatum and miR-124 NPs in the lateral ventricle ('healthy miR-124 NP' group); mice injected with 6-OHDA in the striatum and saline in the lateral ventricle ('6-OHDA saline' group)		This mouse model for PD (10 μg 6-OHDA in the striatum) was chosen based on the following parameters: reduced SVZ neurogenesis (approximately 40% reduction in DCX⁺/BrdU⁺ cells in the SVZ), dopaminergic degeneration (approximately 50% dopaminergic death in the substantia nigra), functional motor deficits, and low mortality rates. MiR-124 NPs were not able to alter the total number of DCX⁺ and DCX⁺/BrdU⁺ cells in the SVZ of both healthy and 6-OHDA-treated mice compared to the respective saline groups. The levels of proliferating neuroblasts (DCX⁺/BrdU⁺ cells) in 6-OHDA-treated mice were approximately 50% lower than in healthy mice. In healthy animals, a significant increase in the number of DCX⁺ and DCX⁺/BrdU⁺ cells was found in the GCL of mice treated with miR-124 NPs as compared to saline animals. MiR-124 NPs increased the number of DCX⁺ and DCX⁺/BrdU⁺ cells in the GCL of 6-OHDA-treated mice as compared to miR-124 NP treated healthy mice. This suggests that miR-124 NPs promote an overall increase in migrating neuroblasts in PD mice. MiR-124 NPs proved to ameliorate motor symptoms of 6-OHDA mice monitored by apomorphine-induced rotation test.	MiR-124 NPs enhance brain repair in 6-OHDA-treated mouse model of PD.
α-Synuclein overexpression mouse model					
Thome et al. (2016)	C57BL/6 (wild-type) and miR-155⁻/⁻ mice, male, 8–12 weeks of age were unilaterally injected with 2 μL AAV-SYN vector (4.0 × 10¹² viral genome/mL diluted in sterile PBS) into the right substantia nigra. At 2 and 4 weeks after transduction, animals were anesthetized and ipsilateral substantia nigra were dissected (n = 6/group)	Controls were mice unilaterally injected with AAV-GFP control vector into the right substantia nigra.	By miScript PCR array, the expression of 84 inflammation- and autoimmune-associated microRNAs was examined in the AAV-SYN mouse model of PD. Several of the microRNAs showed enhanced expression at either the early or later time points; however, miR-155 showed enhanced expression at both 2 and 4 weeks. To validate this result, quantitative PCR was performed with TaqMan probes targeted at mature miR-155. At 2 weeks after AAV-SYN administration, miR-155 was upregulated by 30% (n = 6/ group). At 4 weeks, this method showed a trend toward increased expression of miR-155 but did not reach statistical significance (n = 6/group).	In the mouse with a complete deletion of miR-155, the loss of miR-155 reduced proinflammatory responses to α-synuclein and blocked α-synuclein-induced neurodegeneration.	It is suggested that miR-155 has a central role in the inflammatory response to α-synuclein in the brain and in α-synuclein-related neurodegeneration.
L-type calcium channel Cav1.3 knockout mouse model					
Gstir et al. (2014)	Knockout mice for brain L-type calcium channel Cav 1.3 implicated in PD, male, no. N/A, ages N/A. Mice were anesthetized and brains removed	Wild-type mice	Neuro-ncRNA microarray analysis of hippocampus and striata of Cav 1.3⁻/⁻ mice compared with wild-type control animals revealed 5 and 24 differentially expressed ncRNAs, respectively. Of the 29 ncRNAs, 14 can be assigned to known classes of ncRNAs while the remaining 15 ncRNAs represented currently unclassified ncRNA species. Expression of two miRNAs, designated as miR-204-5p and miR-143-3p, was upregulated in the hippocampus of knockout mice compared with wild-type controls.	Both miR-204-5p and miR-143-3p are predicted to target 3′-UTRs of several ion channel mRNAs indicating potential cross-regulatory effects.	Two differentially expressed ncRNAs were assigned as miRNAs (miR-204-5p and miR-143-3p) and target genes involved in calcium signaling, thus suggesting feedback regulation of miRNAs by calcium signaling.

NA: Not available.
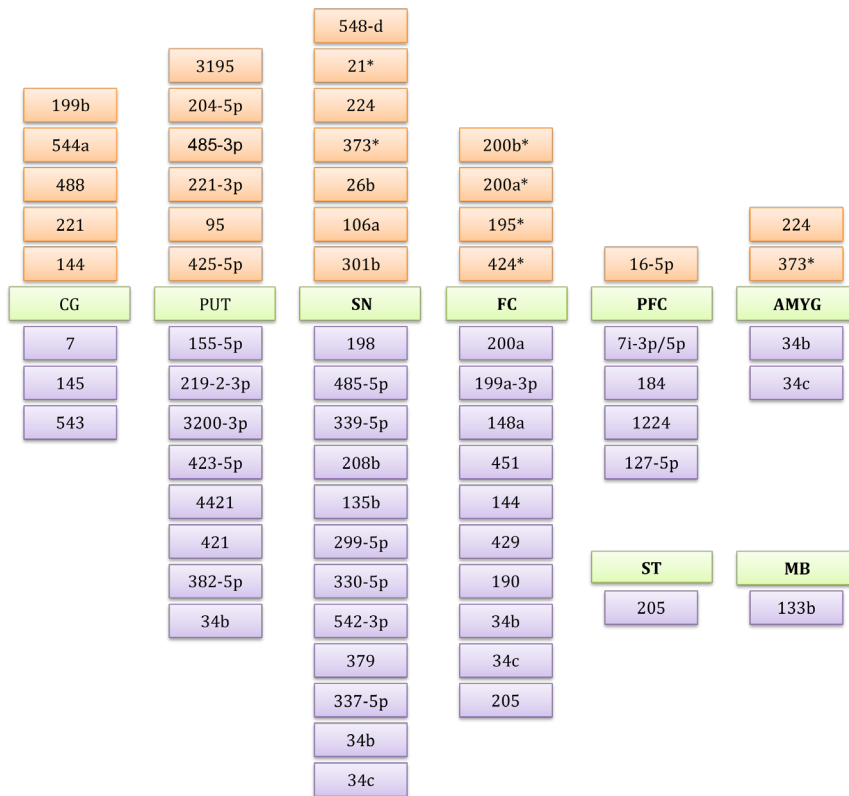
Figure 1 Altered expression of microRNAs in Parkinson's disease brain tissues analyzed (based on the human studies reviewed).
Upregulated levels of microRNAs are shown in orange; downregulated levels of microRNAs are shown in purple. CG: Cingulate gyri; PUT: putamen; SN: substantia nigra; FC: frontal cortex; PFC: prefrontal cortex; AMYG: amygdala; ST: striatum; MB: midbrain.

sociated virus containing miR-205 or by a miR-205 agomir. It was proposed that early downregulation of miR-34b/c in PD triggers downstream transcriptome alterations underlying mitochondrial dysfunction and oxidative stress, which ultimately compromise cell viability (Miñones-Moyano et al., 2011). Upregulation of miR-34b/c may be an applicable therapeutic strategy. Also downregulation of miR-7116-5p in microglia initially potentiated the production of TNF-α in MPTP mouse model, which then amplified the production of other proinflammatory factors to contribute to dopaminergic neuron damage (He et al., 2017). Upregulation of miR-7116-5p could be tested in this model to see if it ameliorated neuron damage. The upregulation of pro-apoptotic Bim mRNA level and protein level induced by MPTP was reduced by miR-124 agomir (Wang et al., 2016). Downregulation of miR-135a-5p in MPTP mouse model was associated with an upregulation in ROCK2 that leads to phagocytosis of dopaminergic neurons (Liu et al., 2016). A miR-135a-5p agomir could possibly protect dopaminergic neurons from phagocytosis. Furthermore, the decreased level of miR-7 (Zhou et al., 2016) possibly contributes to increased α-synuclein accumulation and inflammatory response in MPTP mouse model (Junn et al., 2009). In the animal models of PD, downregulated miR-7 and miR-124 (Junn et al., 2009; Kanagaraj et al., 2014; Wang et al., 2016; Zhou et al., 2016) together with upregulated miR-21 and miR-494 (Xiong et al., 2014; Su et al., 2016) are involved in oxidative stress (Xie and Chen, 2016). In PD patients, downregulated miR-7, -34b/c and -205 (Miñones-Moyano et al., 2011; Cho et al., 2013; Villar-Menendez et al., 2014; Tatura et al., 2016) together with upregulated miR-224 (Alvarez-Erviti et al., 2013) are associated with oxidative stress (Xie and Chen, 2016).

Future Perspectives

Currently the diagnosis of patients with PD is mainly made on clinical manifestations of the disease. Molecular imaging allows a window into the pathophysiology of PD, as well as measuring the severity and progression of the disease. The dopamine terminal dysfunction can be demonstrated using positron emission tomography (PET) or single photon emission computed tomography (SPECT) with different tracers, which contribute to early and accurate diagnosis leading to appropriate medications. PET/SPECT imaging, combined with other individual information such as genetic testing, would assist in providing personalized treatment to improve clinical outcomes and minimize adverse effects (Bu et al., 2016). Neuroinflammation ligand imaging the microglial activation might guide the individualized application of non-steroidal anti-inflammatory therapy in PD patients in the future (Stoessl et al., 2011).

Further large-scale studies of brain tissue samples collected with short PMI from human PD patients are warranted to confirm the changes in microRNA expression that have been reported and to test for gender differences. Where gender was specified, all of the animal studies had used adult male mice at 6 weeks to 6 months of age. Future studies should be performed with aged animals 22 to 24 months of age. Also both male and female animals should be used, as dysregulated microRNA and target gene network related to PD may be gender-specific (Briggs et al., 2015). It has been reported that 50% to 80% of patients with PD have abnormal glucose tolerance that may be further exacerbated by L-dopa therapy (Sandyk, 1993). An observational study concluded that diabetes prevalence was closely similar between patients with PD and subjects without the disease (Becker et al., 2008). In the human studies, it is likely

that many of the PD patients would have been taking medication. Animal models of PD should also incorporate possible medications that could have been used such as L-dopa, antidiabetic, antihypertensive, and antihyperlipidemic drugs. A recent clinical trial has shown that exenatide, a medication used for patients with diabetes mellitus type 2, has the potential to modify PD. Patients with sporadic PD aged 25 to 75 years received subcutaneous injections of exenatide 2 mg or placebo once weekly for 48 weeks in addition to their regular medication, followed by a 12-week washout period. Movement disorder was assessed on a rating scale at 60 weeks. Exenatide had positive effects on motor scores in PD that were sustained beyond the period of exposure. Whether exenatide affected the underlying disease pathology is uncertain (Athauda et al., 2017).

Conclusion

This review has shown the expression of a large number of microRNAs to be altered in brain tissue samples of human PD patients and experimental animal models of PD. Some of these altered microRNAs could serve as potential therapeutic targets since modifying the levels of specific microRNAs was found to have beneficial effects in animal models of PD, with improved functional outcomes. For example, miR-124 agomir delivered to the right lateral ventricle in MPTP mouse model increased the density of TH$^+$ neurons and reduced the upregulation of Bim mRNA level and protein level induced by MPTP, leading to reduced apoptosis (Wang et al., 2016). The predicted downstream targets of many of the dysregulated microRNAs have also been identified, and these included LRRK2, LAMP2A, ROCK2, and several ion channel mRNAs. Inflammation and oxidative stress are considered to be chief mediators of PD, with NO playing a key role in the pathogenesis of this neurological disorder. The biological actions of some of the important altered microRNAs may be in regard to these mechanisms.

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